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(Year 3)

White cabbage: reducing losses from internal disorders and improving supply

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AUTHENTICATION

I declare that this work was done under my supervision according to the procedures described herein and that this report represents a true and accurate record of the results obtained.

Signature…….

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PRACTICAL SECTION FOR GROWERS

Objectives and background

Two thousand seven hundred ha of white cabbage, valued at approximately £20m pa, are grown for storage each year in the UK. The crop represents a significant investment and risk over a period of 18 months of growing and storage before it is sold. Losses from various storage disorders, which are only infrequently evident at harvest, vary from year-to-year. In most years MAFF record losses of 10% of the crop but on occasions much higher losses occur. In the last few years several major co-operatives and growers have recorded complete loss of stored material (up to 600 tons in one store) with others recording substantial losses in the range of 20-80%. This is compounded by substantial buying of cabbage from abroad at short notice at prices of 2 to 3 times higher than offered for the contracted UK crop. For commercial reasons the extent of this is not revealed but clearly unreliability of supply is a major problem. Crop loss is incurred when the extent of the disorders evident in the head on inspection would make it uneconomic to process it. Crops are accepted by processors having a certain proportion of heads or areas of tissue within a head affected provided this does not i) incur excessive handling costs on the line, ii) substantially reduce product recovery on shredding or iii) reduce shred length, an important quality attribute with retail customers. The unreliability of product supply also makes it difficult to match product output and labour requirement with retail customer demand so influencing the processors profitability. High incidences of disorders also increase the costs of waste removal to the processors.

The presence in the internal tissues of necrotic spots (so-called cigar burn), necrotic areas on specific leaves in the phyllotaxis and usually at the margins (so-called tip-burn) and pepper spot are commonly occurring disorders. These problems are not usually evident in cabbage heads at harvest and at store loading.

Cigar burn has been associated with the infection of plants by turnip mosaic virus (TuMV) and cauliflower mosaic virus (CaMV). Tip-burn and pepper spot have been attributed to deficiency in the local supply of calcium associated with inadequate transport and with calcium metabolism although other cation deficiencies are implicated in pepper spot. There appear to be interactions between calcium supply and virus infection recorded, but not verified, in other crops in relation to tip-burn and we have recently recovered CaMV from stored cabbage heads displaying tip-burn symptoms but no cigar burn symptoms.

Factors inducing calcium deficiency include: a) high leaf expansion rates due to high temperature causing high demand for calcium and to high N and K levels; b) high transpiration may increase calcium import by the outer at the expense of inner

leaves. Conditions in the leaf during storage may affect calcium re-distribution or calcium metabolism.

It is not known currently how to produce a crop free from these disorders which will store reliably and there are no reliable methods of pre-storage assessment of storage potential in relation to occurrence of disorders. Pictures of these disorders are given in the Appendix (Figures 18 and 19).
The aim of the project is to reduce the impact on the efficiency and profitability of production and preparation of storage white cabbage used for processing from the unpredictable occurrence of internal disorders by:

- identifying the causes
- developing agronomic techniques and breeding selection procedures
- developing and introducing physiological and virological pre-storage tests to inform decision making strategies

The scientific objectives are:

1) to identify the role of the three most prevalent viruses (TuMV, CaMV and beet western yellows (BWYV) and mixtures of these in internal disorders during storage;

2) identify the role of calcium supply, transport and metabolism on internal tip-burn and relate this to factors which influence growth rate and to storage environment and its duration;

3) determine if Ca^{2+} transport and Ca^{2+} status of leaves is affected by virus infection;

4) devise growing techniques to reduce the incidence of disorders; develop improved field-based selection techniques to screen germplasm for resistance to Ca^{2+} and virus induced disorders;

5) develop diagnostic tests, including RT-PCR or immuno-capture RT-PCR-based methods to detect viruses for predicting storage potential at the time of harvest.

SUMMARY OF RESULTS

1. Cigar burn was associated with TuMV infection and exacerbated by CaMV in joint CaMV + TuMV infections. Late infection increased the incidence and severity of cigar burn symptoms, especially when plants were also infected with CaMV. Data from a hydroponic experiment suggested that CaMV might cause cigar burn symptoms. This effect was not seen in the soil grown plants so it may have been an artifact of the hydroponic system.

2. Tip-burn was associated with BWYV infection and exacerbated by CaMV in joint BWYV + CaMV infections. There was evidence that tipburn symptoms induced by BWYV may increase over time in store. There were also indications that the incidence and severity of BWYV-induced tip-burn may be reduced by concurrent TuMV infection.

3. As in year 2, BWYV and CaMV infection caused significant yield reductions, whereas TuMV infection did not. Late infection with CaMV, produced significantly greater yield loss than early infection. In hydroponics virus-induced yield losses were affected by calcium availability. Where calcium levels were maintained throughout the growth period, no virus treatments significantly affected yield. Yield losses occurred only where calcium levels were allowed to fall.

4. ELISA techniques for detection of BWYV and TuMV are capable of detecting virus infections prior to harvest. The PCR-based detection system devised for CaMV is also capable of detecting infection prior to harvest and generated a result faster than inoculation to a susceptible host. It is possible to quantify results from both ELISA and PCR. Quantification of CaMV levels would not be possible using the inoculation method.

5. Deliberate subjection of cabbage plants to a period of drought followed by ample re-watering resulted in no incidence of internal tipburn at harvest or after 7-months storage.

6. Calcium supply to the interior of the head was also unaffected by drought treatment.

7. Covering plants with polythene to decrease the rate of transpiration resulted in less calcium in the innermost region of the head.

8. Symptoms of internal tipburn can be produced in cabbage plants grown in hydroponics even with an ample supply of calcium. The calcium concentration in heads of hydroponically grown cabbage is much lower than that of soil-grown heads.

ACTION POINTS FOR GROWERS:

- Cigar burn appears to be due to turnip mosaic virus infection (see Appendix figure 18).

- Tip-burn appears to be associated with beet western yellows infection (see Appendix figure 19).

- Both cigar burn and tip-burn symptoms are exacerbated by cauliflower mosaic virus infection.

- Infection in June compared with infection in April (pre-transplanting) produced greater levels of cigar burn.

- Control of turnip mosaic virus and to a lesser extent cauliflower mosaic virus should reduce the potential for cigar burn symptoms to develop during storage.

- Control of beet western yellows virus and to a lesser extent cauliflower mosaic virus should reduce the potential for tip-burn symptoms to develop during storage.

- Further work is required to examine the effect of time of infection by beet western yellows virus on the severity of tip-burn symptoms.

- Beet western yellows virus and cauliflower mosaic virus caused significant reductions in harvested head weight.

- An insufficient supply of calcium to the head can lead to internal tipburn in hydroponics. Thus, maintaining even, steady growth throughout the season is desirable to minimize the risk of internal tipburn.

- In field experiments smaller heads as a result of closer spacing than that used in current commercial practice gave a lower incidence of internal disorders. Modifying spacing may provide an approach to reducing internal disorders.

SUMMARY OF PROGRESS

Objective 2
Physiological studies

Milestones

	Completed **Not completed**	**√** **x**
Year 3 crop April 1999	Establish plants in pot/ Hydroponic system using $^{45}Ca^{2+}$	x
Year 3 crop April 1999	Establish plants in polytunnel ('field') experiment	√
Year 3 crop June 2000	Completion of assessment of disorders after storage	√

It was agreed, on Health and Safety grounds, to abandon $^{45}Ca^{2+}$ studies to identify flow of Ca^{2+} to different leaves in favour of a Ca^{2+} budget by chemical analysis.

Objective 1
Virological studies

Year 3 crop	Investigate techniques for virus Detection	√
Year 3 crop June 2000	Completion of assessment of disorders after storage	√

Objective 3
Integrated virological/physiological studies

Year 3 crop April 1999	Establish plants in hydroponics to study influences of virus infection on Ca^{2+} status and transport and internal disorders	√
Year 3 crop June 2000	Completion of assessment of disorders after storage	√

		Completed	**√**
		Not completed	**x**

Objective 4
Agronomic and screening studies

Year 3 crop April 1999	Establish systematic spacing design experiment to obtain 'head size/internal disorder' response curves		√
Year 3 crop June 2000	Completion after storage of assessment of disorders		√
Year 1,2, 3 & 4 crops from industry	Collect protocol/disorder information from companies	Ongoing	

EXPERIMENTAL SECTION
OBJECTIVE 2
PHYSIOLOGICAL STUDIES
Material & Methods

Polythene tunnel experiment 1999

Cultivation of these plants was based on the same industry protocol as used in 1998.

Dutch White cabbage seed (cv. *Impala*) was sown into Hassy trays on 24 March and raised in a polythene tunnel before planting out on 25 May into soil (a sandy loam of the Wick Series) in two fan-ventilated polytunnels. Plants were spaced at 60cm within and between rows. In each tunnel there were 42 rows of 11 plants. Allowing for guard rows between plots and at the sides, this provided 45 plants per plot for sampling. The crop in each tunnel was split into three blocks along the length of the tunnel to offset effects of end-to-end temperature gradient. Within each block the treatments were randomly allocated to one of two plots.

Irrigation was applied by ramjet hose laid along each row of 11 plants. All plots were provided with the same quantity of water until the plants had substantial heads (average fresh weight just under 2kg). At this time (23 August) water was withheld from a “droughted” treatment until 20 September, when watering was resumed on these plots. Watering was maintained on the control plots throughout the experiment. At the beginning of the period of drought, two plants on each plot were enclosed in polythene tents. The enclosures were removed on 5 and 6 October. Soil water status was measured using a neutron probe and demonstrated clear differences between the treatments (Figure 1).

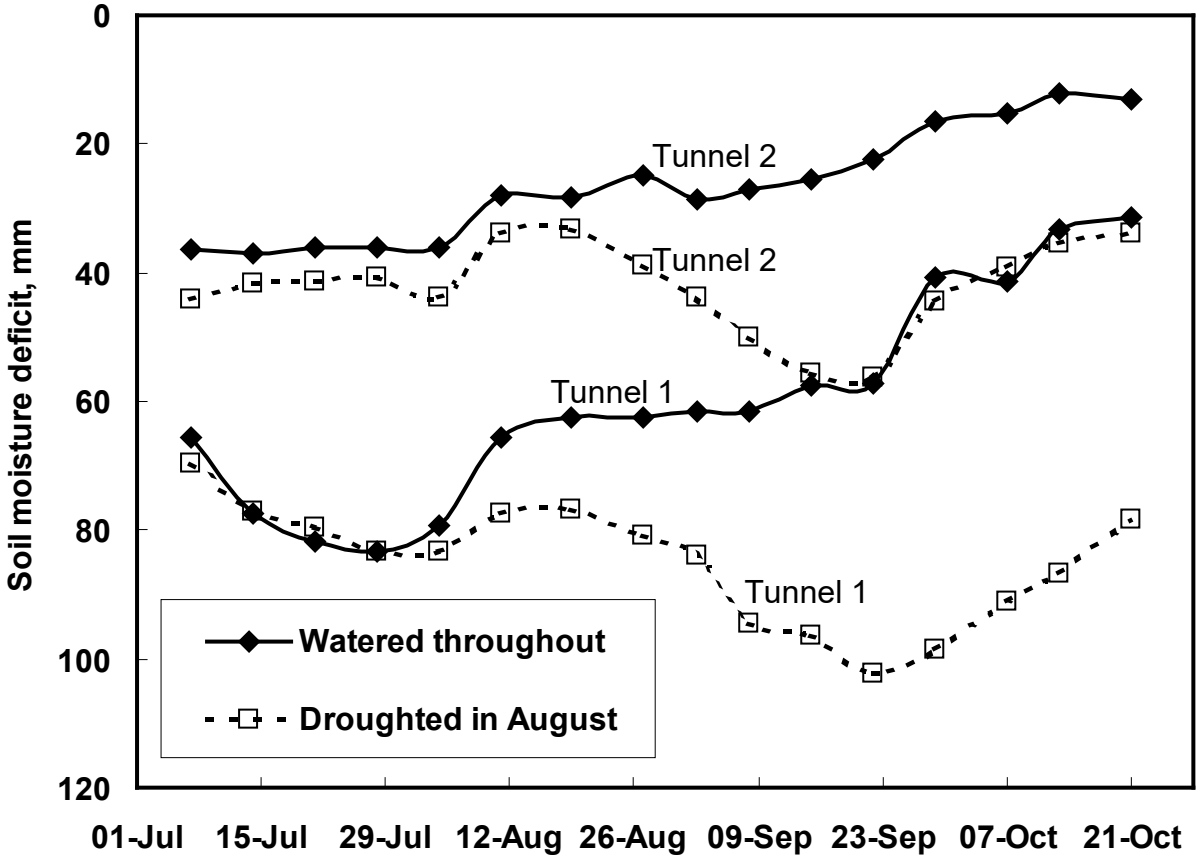


Figure 1. *Moisture deficits for 600mm depth of soil from different irrigation treatments in the 1999 polythene tunnel experiment*

All plots received a base dressing of 170 kg ha^{-1} nitrogen before planting and a top dressing of 216 kg ha^{-1} on 17 August.
Plants were sampled on 9 July, 18 August and 4, 5 and 6 October to monitor growth and development. On the latter two occasions samples for calcium analysis (and other mineral nutrients) were taken from various parts of the plant to establish

1) the distribution of calcium in cabbage plants and
2) the effect on calcium distribution of disruption to growth and water movement by droughting and enclosure.

On 2 November 40 heads were cut from each plot and sprayed with a mixture of Rovral and Ridomil MBC as per commercial specification. The heads were placed in nets for future handling and then into commercial bins. The bins were transported to storage at 1°C in a commercial sealed store in which oxygen was reduced to c. 17% and carbon dioxide concentration remained at 3%. On 4 November, samples of head tissue were taken from one of the remaining plants on each plot for calcium analysis.
Assessment of the stored heads for the presence of internal browning/necrosis disorders was done on 6 March and 6 June 2000, when 20 heads from each plot were removed from store on each occasion.
Samples were taken from 5 heads per plot for estimation of calcium concentration.

Calcium: method of analysis
A sample of approximately 4g fresh weight of tissue was taken and shredded, oven-dried and weighed again. It was then ashed at 550°C and the residue digested until dry in 2ml of 50% HCl on a hotplate at 160°C. This was then dissolved in 10ml of 50% HCl and analysed by Inductively Coupled Plasma (ICP). Concentrations of calcium, magnesium, phosphorus, iron, zinc, manganese, copper, boron and sulphur were measured. Concentrations of total mineral ash and of nutrients in plant tissue were expressed on a dry matter basis.

Anatomical studies
Seed of cvs Polinius, Bentley and Impala were sown into modules on 7 July 1999 and transferred to pots on 2 August. On 9 September the fourth leaves of similar size were removed from 15 plants of each variety and placed into sealed plastic bags. These were stored in a cold room. A sample (approx. 1 cm long) of whole petiole was cut from just below the lamina. These sections were frozen in liquid nitrogen vapour for 5 minutes, immediately placed into disposable scintillation vials and stored at –20°C. Anatomical sections were prepared by embedding the samples over liquid nitrogen in Tissue-Tek. These samples were then sectioned at 24 microns thickness using the freeze-microtome. The sections were air-dried and stained for 1 hour with Auramine O-SO2. This selectively stains for lignin and so highlights xylem vessels for microscopy. Sections were then washed to remove excess stain and air-dried.

Results and Discussion

Polythene tunnel experiment 1999

The purpose of this experiment was to examine the relationship between the incidence of internal necrosis (tip burn) in stored cabbage heads with the imposition of conditions leading to rapid growth rate. To achieve this the plants were subjected to a period of drought followed by a period of plentiful water availability. Manipulating growth rate was intended to induce an imbalance between growth and calcium supply to rapidly growing parts of the plants. Attempts were also made to alter the rate of transpiration and water status of the plants by enclosing some of them in polythene tents to raise the surrounding relative humidity. It was hoped that this would further disturb the supply of calcium to growing heads.

Of 475 heads assessed for the presence of internal necrosis after storage for five and eight months there were only 9 heads with symptoms which could be described as tip burn. Not surprisingly, with such small incidence there was no significant association with the drought and re-watering treatment imposed. Also, symptoms were not observed in any of the heads enclosed in a polythene tent.

Comparison of calcium distribution with magnesium distribution (Figure 2) shows that heads have less calcium than they do magnesium relative to rest of the plant.

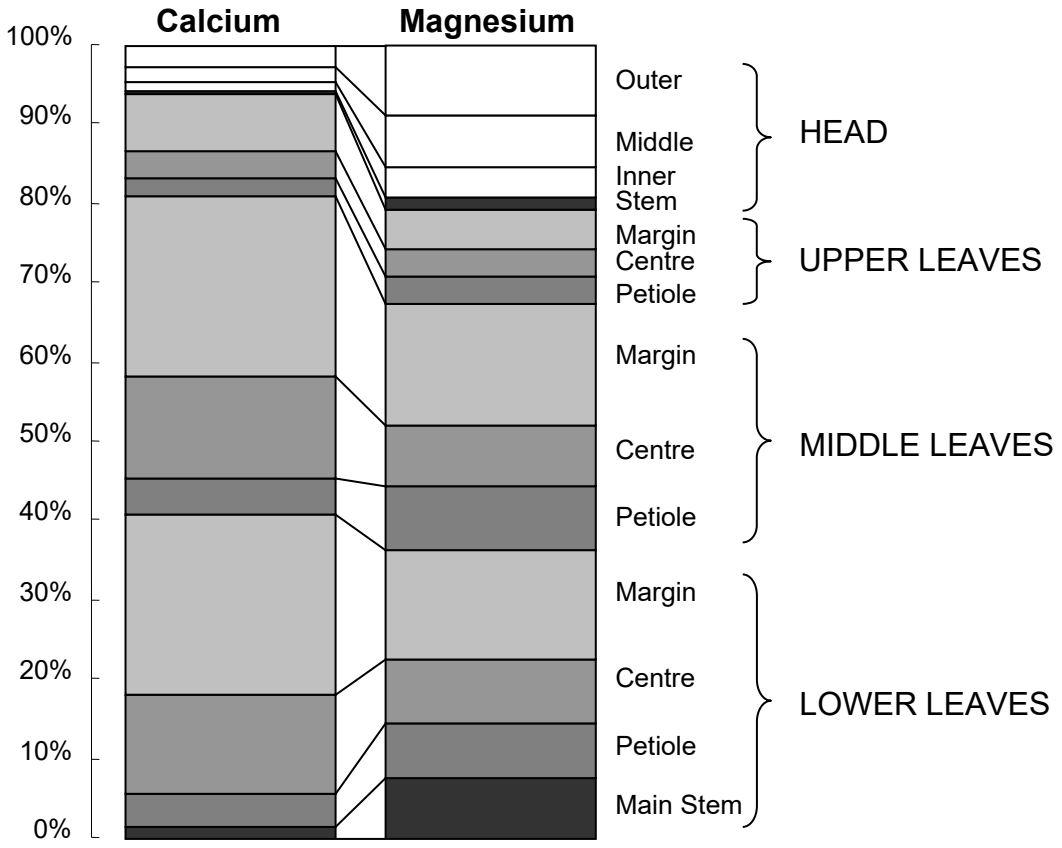


Figure 2 Distribution of calcium and magnesium in cabbage. Polythene tunnel experiment, 1999. August sample.

With respect to distribution in the head, other nutrients and dry matter content behave like magnesium. Comparison of calcium concentration in various parts

of the plants (Figure 3) clearly illustrates that concentrations in the head are much lower than in other parts.

Drought and re-watering had no effect on concentration of calcium (per unit dry matter) in the head or on distribution in the plant immediately after completion of treatment nor at final harvest (Figure 3). Covering the plants with polythene resulted in a **smaller** concentration in the inner head zone compared with uncovered plants, but greater concentrations in the outer zones of the head, the head stem and central region of upper leaf lamina (Figure 3). Concentrations of magnesium in all zones of the head (except the stem), in all upper leaf parts and the lamina of middle leaves were greater in plants covered with polythene (Figure 4). For phosphorus (not illustrated) covering with polythene resulted in increased concentrations in all leaf lamina and in the outer and middle zones of the head. Generally, for all other nutrients measured, polythene enclosure resulted in an increased concentration of the nutrient concerned, where there were significant effects.
Dry matter content was less in all parts of the plant as a result of enclosure, with the exception of the inner head zone where it was greater. This elevated level of dry matter could have been responsible for the decreased calcium concentration. However, no similar effect was observed for any other nutrient. Enclosure with polythene increased the mineral ash content per unit dry matter principally in stem and petiole tissue and the outer and middle zones of the head. Thus the decrease in calcium in the inner head zone was not associated with a general reduction in the concentration of mineral nutrients.

At final harvest, mean calcium concentration of head tissue was 0.37%±0.029. This was considerably lower than mean calcium concentrations during August and October (respectively 0.48%± 0.026 and 0.58± 0.046). Analyses of samples from stored heads are in progress.

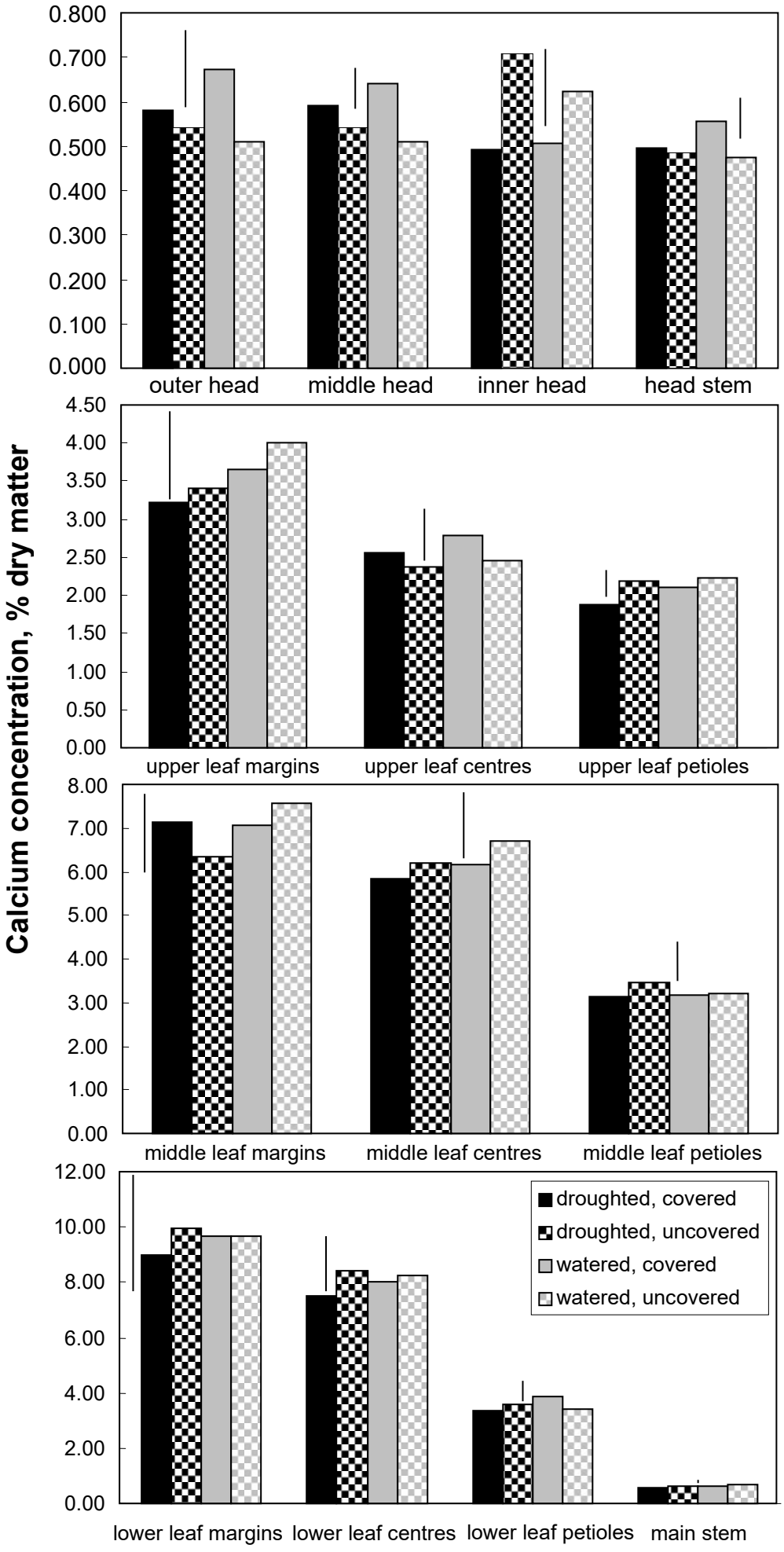


Figure 3. Effect of drought and polythene enclosure on calcium concentrations in cabbage parts. Polythene tunnel experiment 1999.

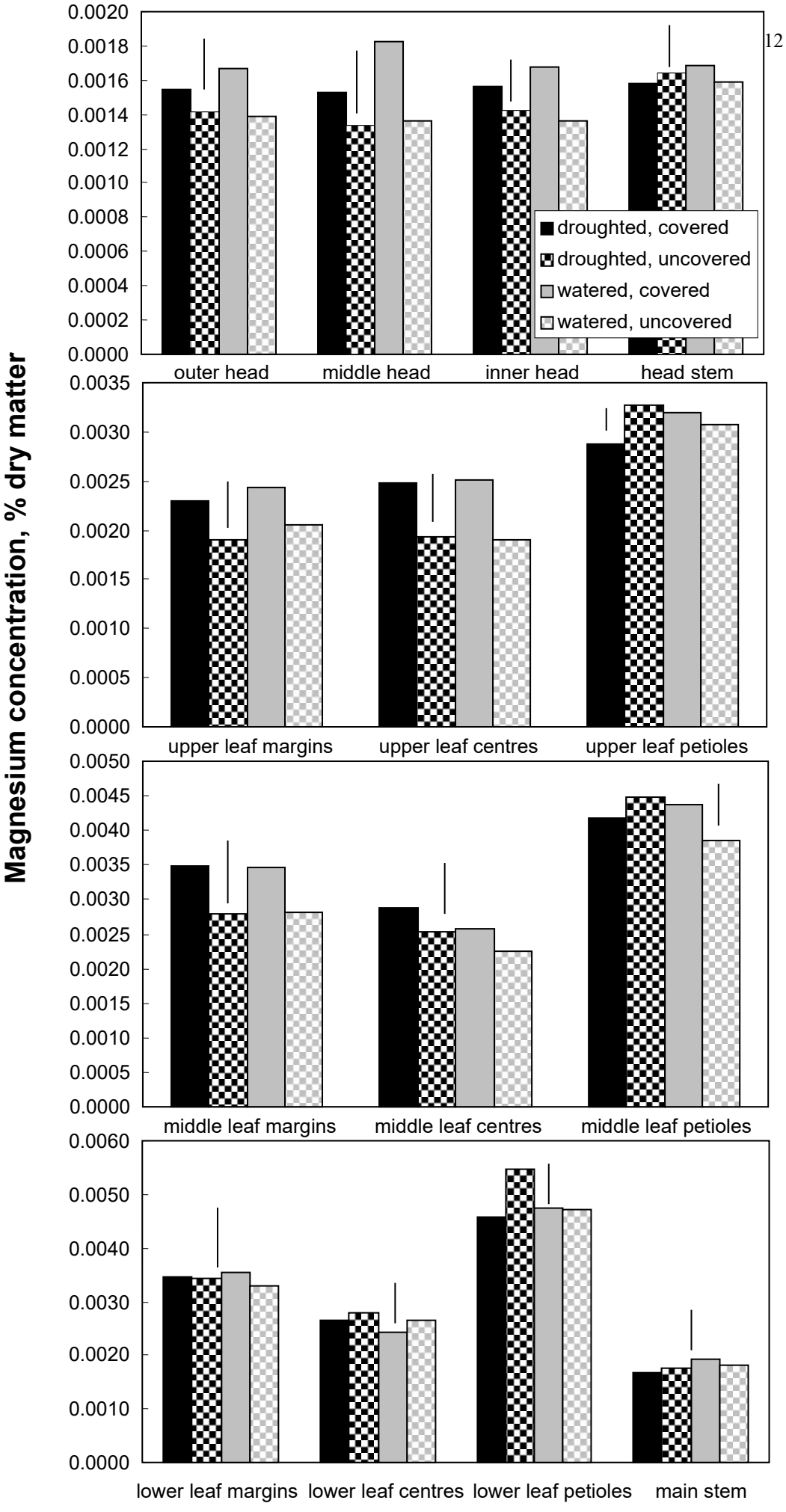


Figure 4. Effect of drought and polythene enclosure on magnesium concentrations in cabbage parts. Polythene tunnel experiment 1999.

Anatomical studies
Xylem vessels in petioles of leaves are being examined, because these conduct water through the plant and are thus also responsible for the distribution of mineral nutrients. Calcium is transported only in xylem vessels. Other nutrients can be re-distributed via phloem elements, which also provide the principle route for transport of organic compounds. Differences in the amount of xylem or in its composition (e.g. many small vessels versus a few large ones) may contribute to differences in transport of nutrients, particularly calcium. We are examining xylem tissue in the three varieties so far used in this project to determine whether fundamental structural differences are present. The potential value of this approach would be its suitability as a screen for breeding material.

The samples are presently being sectioned and stained (Figure 5). When this has been completed, the number and area of xylem vessels will be estimated and the means for the varieties compared.

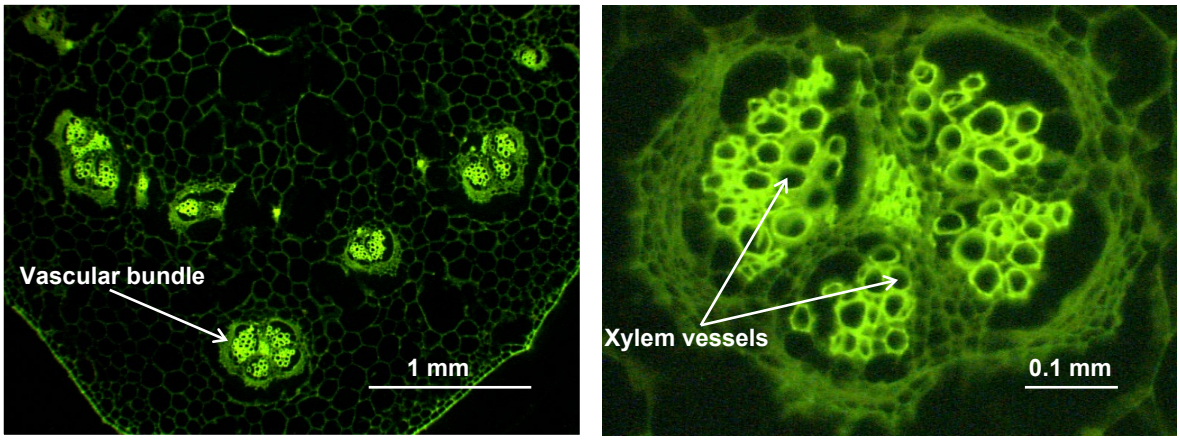


Figure 5. Cross section through petiole of Dutch white cabbage at low and high magnification, illustrating fluorescence of xylem vessels stained with auramine O-SO_2.

Hydroponic experiment 1998/1999
Practical details and some preliminary results from this experiment were provided in the Report for the second year. The purpose of the experiment was to examine whether an association between the incidence of internal necrosis in cabbage heads and withdrawal of calcium from the nutrient solution could be observed. When last year's report was written, heads stored until August were not included and the available data had not been subjected to statistical analysis. A summary of the analysed data is presented here.
Mean scores for internal necrosis were increased only by calcium withdrawal for a 30-day period (Table 1). The incidence of plants with no symptoms suggested a small interaction with time of storage ($p<0.05$), but only in that there were fewer of them amongst untreated plants examined after storage (Table 1). The combined results from sampled heads examined immediately after growth and after 3 months' storage showed that there were consistently fewer unaffected

plants among those subjected to a 30-day withdrawal period than in the other treatments (P<0.001). There was no difference in the response of the two varieties with respect to these results. There was, however, a suggestion that for cv. Polinius the number of plants with more severe scores was greater (p<0.05) than for cv. Impala (Table 1).

Table 1. The effect of calcium withdrawal on the incidence and severity of internal necrosis in cabbage heads from hydroponically grown cabbage, 1998/99 experiment. (Year 2)

Treatment	Mean score	Incidence of zero scores, %			Incidence of scores 2 and 3, %	
		1/5/99	9/8/1999	All heads	Polinius	Impala
C	0.52	82±9.1	55±13.5	71±7.8	14±9.2	15±9.6
15E	0.24	70±11.7	100±0.2	83±6.6	0±0.1	14±9.0
15L	0.43	78±10.6	71±15.8	75±9.1	25±11.8	0±0.1
30	1.27	37±12.7	32±12.8	35±9.1	52±12.2	19±11.4
lsd (5%)	0.555					

C= calcium concentration maintained (control), 15E = calcium withdrawn for 15 days from 9/3/99, 15L = calcium withdrawn for 15 days from 16/3/99 and 30 = calcium withdrawn for 30 days from 9/3/99

Calcium concentration in head tissue was significantly less in plants which were subjected to a 30-day period of withdrawal and the effect seemed to be greater for cv. Polinius than cv. Impala (Table 2). Comparison of calcium concentrations for plants with differing levels of symptom score showed a negative association of symptom score with calcium concentration.

Table 2. The effect of calcium withdrawal on the concentrations (% dry matter) of calcium and magnesium in cabbage heads from hydroponically grown cabbage, 1998/99 experiment (Year 2).

Treatment	Calcium		Magnesium
	Impala	Polinius	
C	0.127^{a}	0.145^{a}	0.159^{a}
15E	0.111^{ab}	0.096^{b}	0.191^{b}
15L	0.116^{ab}	0.078^{bc}	0.183^{bc}
30	0.059^{c}	0.051^{c}	0.167^{ac}
lsd (5%)	0.0038		0.022
Symptom score			
0	0.107^{a}		0.171^{a}
1	0.081^{b}		0.173^{a}
2	0.095^{ab}		0.189^{a}
3	0.073^{b}		0.185^{a}
lsd (5%)			
0 vs 1	0.023		0.030
0 vs 2	0.036		(average)
0 vs 3	0.028		

Key as for Table 1. Means with the same post-scripted letter are not significantly different.

OBJECTIVES 1 and 5
VIROLOGICAL STUDIES

Materials & Methods

Seedlings of two cultivars (Polinius & Impala) of white cabbage were sown in modules on 10 March 1999 and grown initially under glass then transferred to insect-free gauze houses on 7 April. The experiment was conducted with cultivar Impala since this represented currently grown cultivars better than cultivar Polinius. The Polinius seedlings were inoculated with TuMV only and used as a positive control for TuMV-induced cigar burn.

The treatments applied to cultivar Impala were as follows:
beet western yellows virus (BWYV) early inoculation only,
turnip mosaic virus (TuMV) early and late inoculations,
cauliflower mosaic virus (CaMV) early and late inoculations,
BWYV + CaMV early inoculation only,
BWYV + TuMV early inoculation only,
TuMV + CaMV early and late inoculations.

Early inoculations

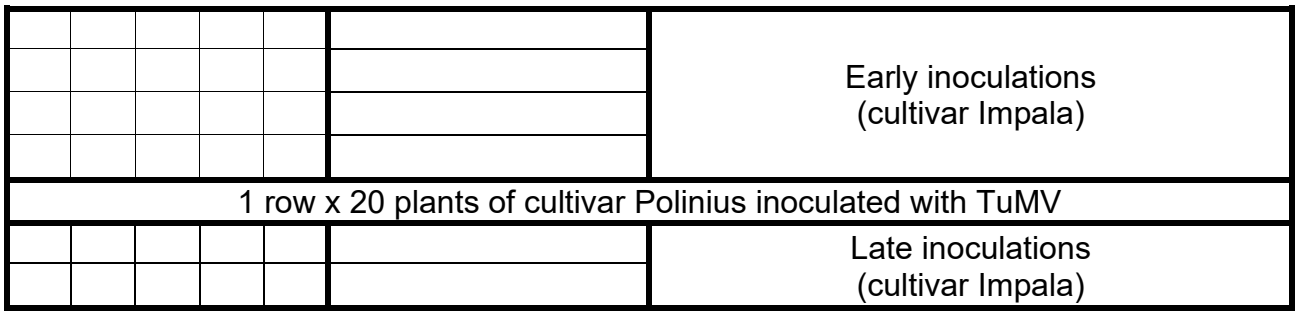
BWYV is not mechanically transmissible therefore plants were infected with this virus using aphids on 4 May and the aphids subsequently killed by treatment with Dovetail on 11 May. The seedlings were then mechanically inoculated with TuMV on 12 May and with CaMV on 13 May. Uninoculated control plants were also included in the experiment. Dursban was applied to the seedlings as a pre-planting drench to prevent cabbage root fly infestation, followed by an application of Spannit granules on 19 May (after transplanting).

Late inoculations

Plants were inoculated on 23 June (CaMV) and 25 June (TuMV). The date for the inoculations was chosen to represent the most likely times of the major aphid migration. BWYV was not included in the late inoculations since the virus requires an aphid vector for transmission and introduction of aphids into the experiment involved too great a risk of cross contamination.

A nutrient analysis of the soils in the Tygan houses used for growing the transplants was made. The soils were fertilised with nitrogen at either 90.4 kg ha^{-1} (house 1) or 180.7 kg ha^{-1} (houses 2, 3 and 4) (based on the analysis) on 23 April, raising concentrations of nitrogen in the soil to commercially acceptable levels. No additional potassium or phosphate was required. The seedlings were transplanted into the insect-proof Tygan houses on 14 May. Plants were spaced 60 cm apart with a total of 140 plants in each of 4 houses. (Figure 6).
Each house contained two sections of cultivar Impala plants separated from each other by a single row of 20 cultivar Polinius plants inoculated with TuMV. Each of these 2 sections was divided into 2 plots (4 plots per house).

Figure 6. Planting layout



5 plants / row

1 row / treatment

8 treatments / large plot
(6 virus and 2 uninoculated)

4 treatments / small plot
(3 virus and 1 uninoculated)

2 large plots / house

2 small plots / house

Applications of Toppel and Dovetail to prevent aphids were applied alternately at fortnightly intervals, Bravo was applied monthly to prevent *Alternaria* and *Botrytis* and three treatments of Fubol were applied during the season to prevent white blister. Nuvan and dichlorvos were applied as required throughout the growing period to control thrips. A top dressing of nitrogen at 228kg ha^{-1} was applied to all houses on 28 June.

The growth of plants and appearance of symptoms were recorded at weekly intervals. The plants were tested for the presence of the viruses 3 weeks prior to harvest. BWYV was readily detectable from cabbage leaves by triple antibody sandwich ELISA. A triple antibody sandwich procedure was developed to detect TuMV from cabbage leaves. This method made the grinding of samples in liquid nitrogen to extract the virus (which was required in the previously used modification of the plate trapped antigen protocol) unnecessary and gave better detection of TuMV than the previous protocol. Detection of CaMV directly from cabbage leaves was not achieved by ELISA. Consequently this virus was assayed for by inoculating to susceptible indicator plants (Mustard cv. Tendergreen). The presence or absence of the virus in the mustard plants was then determined by ELISA.

The cabbage heads were harvested on 1 November following commercial guidelines, weighed and placed in nets, each net containing heads from a single row (a single treatment). Heads were then drenched to run-off with Rovral and Ridomil, again according to commercial practice, prior to placement in cold storage. The heads were separated into 2 batches, one for assessment after approximately 4 months and the other after 7 months in store.

The first heads were removed from the cold store and assessed on 6 March 2000 (18 weeks post harvest). Head weight was recorded and the external fungal growth on the head noted. Heads were cut into quarters and

assessments of internal symptoms from diagonally opposite quarters of the heads were made. The external fungal growth, internal tip-burn symptoms and internal cigar burn symptoms were recorded on four point scales (Table 3). Samples were taken from each head for virus testing. A further assessment was made, in a similar manner, on 6 June (31 weeks post harvest).

Table 3. Scoring regime

Cigar burn
0 No symptoms
1 One spot to a few spots on ≤25% of leaves
2 Spots on >25-50% leaves or severe spots on >10% of leaves
3 Spots on >50% of leaves, or severe spots on >25% of leaves

Tip-burn
0 No symptoms
1 0 – 5% of leaves with tip-burn
2 5 – 10% of leaves with tip-burn or 0 – 5% of leaves with severe tip-burn
3 >10% of leaves with tip-burn or 5 – 10% of leaves with severe tip-burn

External fungus
0 No external fungus
1 Slight amount external fungus over part of head
2 Light fungus infection over all of head or heavy infection with tissue damage over part of head.
3 Heavy fungus infection over most of head with significant tissue damage

Results and Discussion

Symptoms during growth

Two main types of external symptom were observed during the growth of the plants: vein clearing (which progressed to inter-veinal chlorosis as plants matured) and necrotic spotting. The latter symptom appeared to be associated with TuMV treatments whilst the vein clearing symptoms were more prevalent in external leaves of CaMV-inoculated plants. Some plants inoculated with a combination of CaMV and TuMV showed both vein-clearing and necrotic spotting in the same plants. The level of symptoms recorded is shown graphically in Figures 7-12. No distinction has been made between the vein–clearing / chlorosis and the necrotic spotting symptoms in these plots.

BWYV-infected plants showed little evidence of symptoms (Figure 7). The few symptoms that were seen were of the vein-clearing type. Symptoms in external leaves of the early TuMV-inoculated plants increased from early July (approximately 2 months post transplanting) and reached maximum levels of approximately 40% (Impala) and 65% (Polinius) of the plants inoculated. (Figure 8). The late inoculated plants (Impala only) showed a similar progression of symptoms to the early inoculated Impala plants, commencing at the same time (early July) and progressing to approximately the same final incidence (37%).

The lag between inoculation and appearance of external symptoms was greatly reduced in the late inoculation. This may have been due to the plants growing more rapidly at the time of the late infection than at the earlier (pre-transplanting) inoculation.

Symptoms in external leaves of the early CaMV-inoculated plants started to appear in early June (approximately 1 month after transplanting) and reached the highest incidence for any of the treatments, with 100% of plants affected (Figure 9). The late inoculated plants began to show symptoms in early July (2 weeks post inoculation). The symptoms progressed at a more rapid rate than in the early inoculated plants, reaching near parity with them (97% incidence) by the beginning of September. Symptoms in the external leaves the late inoculated plants began to appear at the same time in plants inoculated late with TuMV. In contrast, symptoms in external leaves of the earlier CaMV inoculated plants first appeared approximately a month earlier than in plants inoculated early with TuMV.

BWYV in combination with TuMV (early inoculation only) produced a lower maximum incidence of symptoms (27%) when compared with TuMV alone (40%) although symptoms began to appear 2 weeks earlier (mid June) (Figure 5). BWYV in combination with CaMV (early inoculation only) induced a rapid appearance of symptoms (Figure 10). This began at the same time as in those plants inoculated early with CaMV alone, but reached maximum incidence of (90-95%) three weeks earlier. The maximum incidence recorded (95%) was slightly lower than for CaMV alone (100%) (Figures 9 and 11). The combination of CaMV with TuMV caused a rapid increase in symptoms (Figure 12) in external leaves of both early and late inoculated plants, similar to CaMV single virus inoculations, but with maximum incidences of 92% (early) and 97% (late) of inoculated plants. In this treatment, the first necrotic symptoms appeared later than the vein clearing / chlorotic symptoms by approximately 9 weeks (early inoculation) and 5 weeks (late inoculation).

Figure 7. Percentage of BWYV-inoculated plants with symptoms

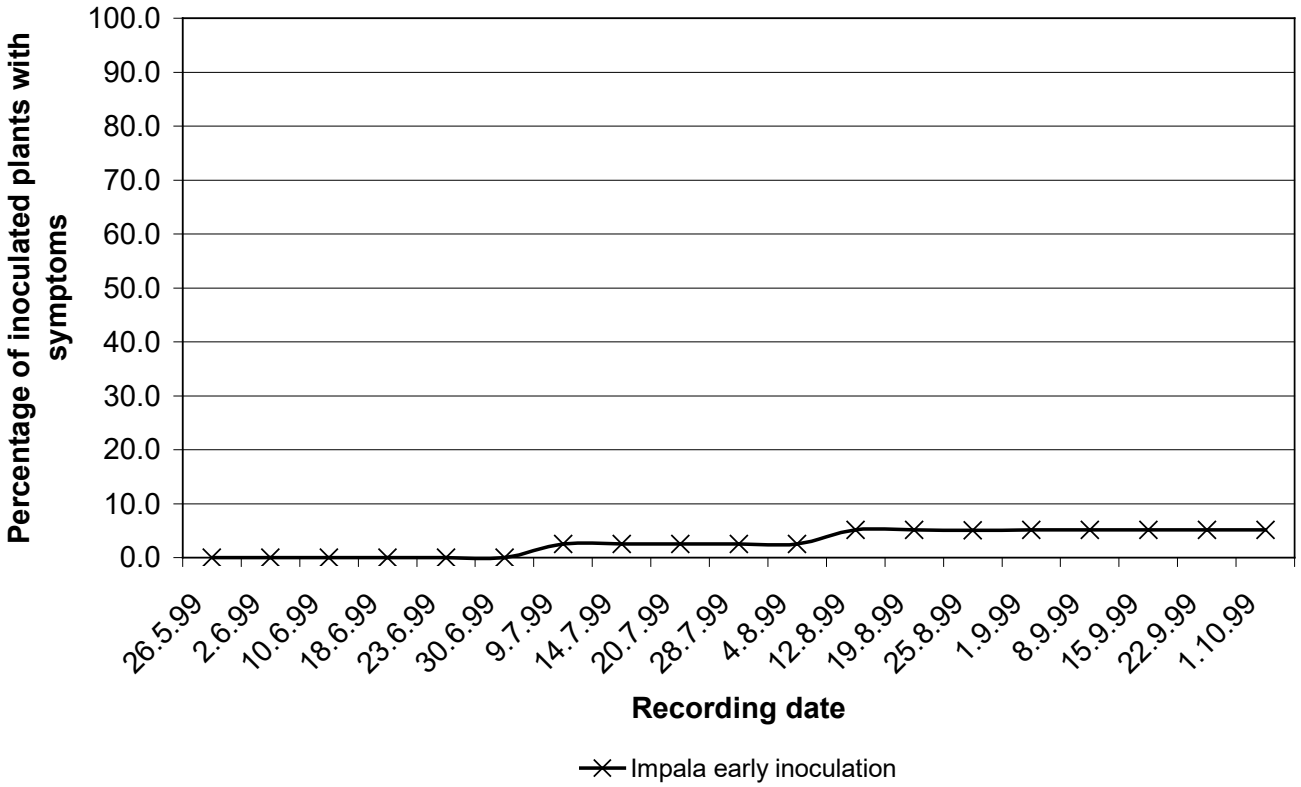


Figure 8. Percentage of TuMV-inoculated plants with symptoms

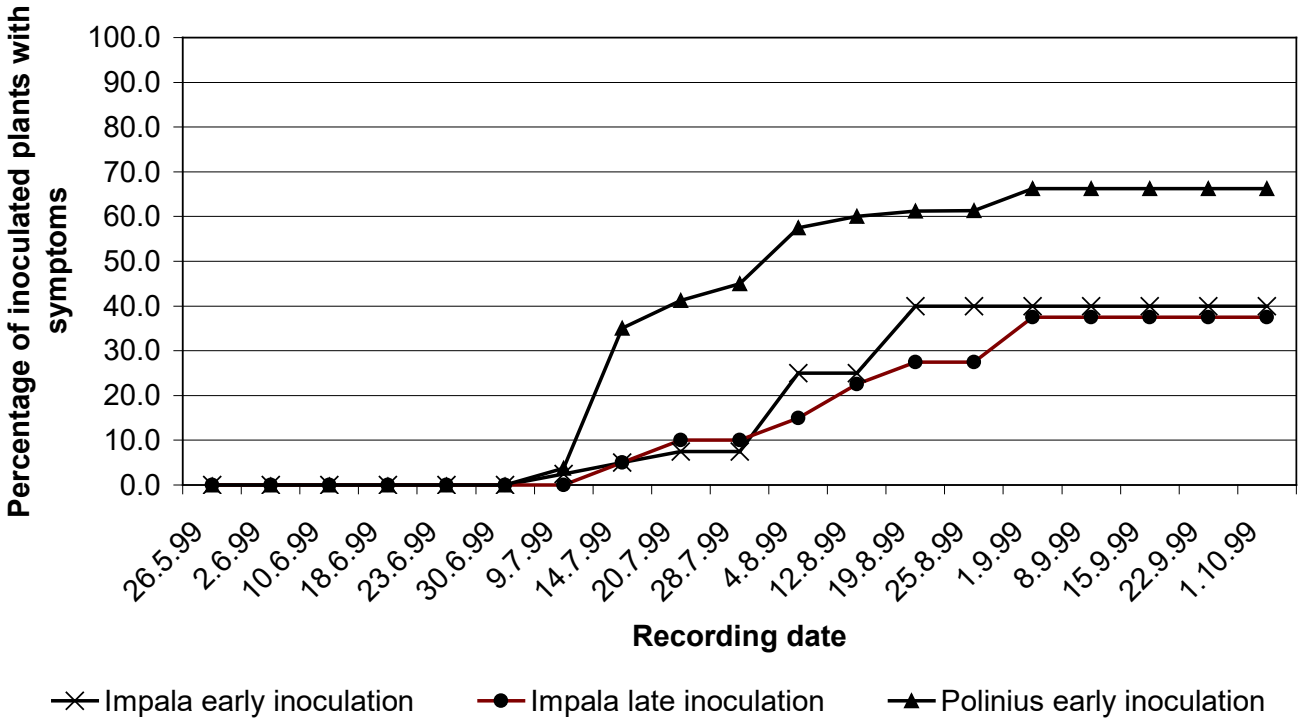


Figure 9. Percentage of CaMV-inoculated plants with symptoms

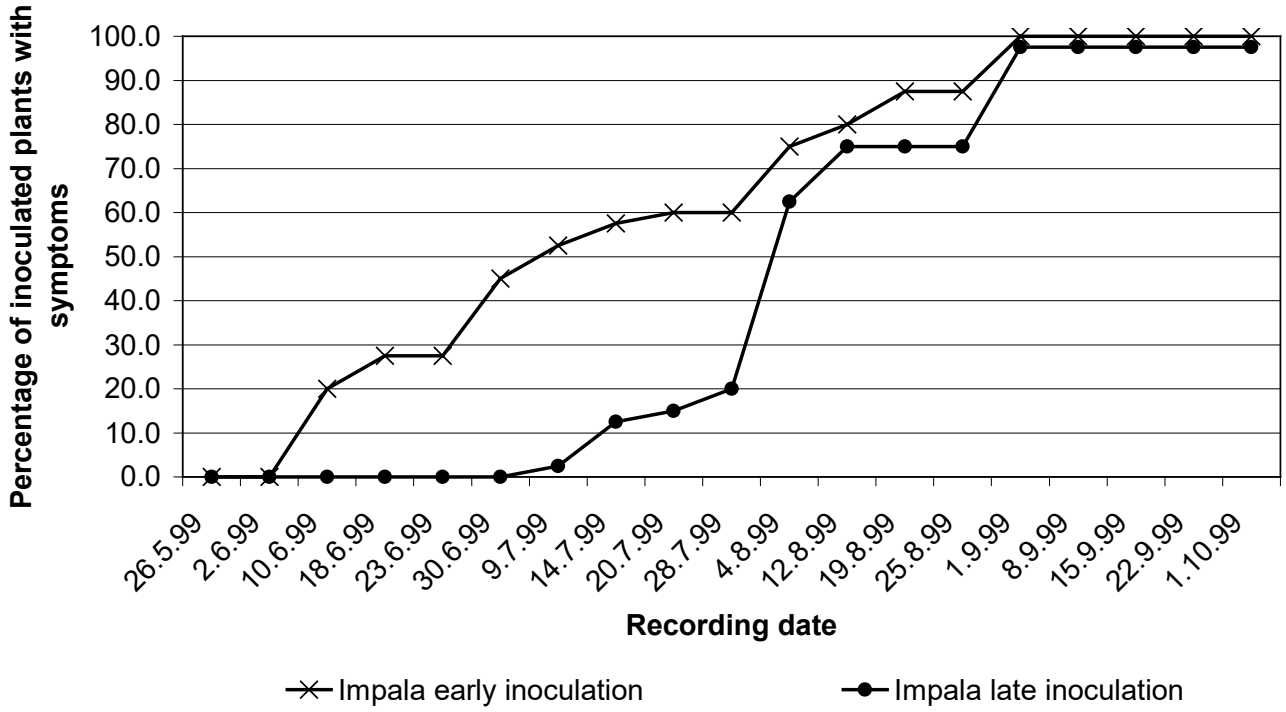


Figure 10. Percentage of plants inoculated with a combination of BWYV and TuMV showing symptoms

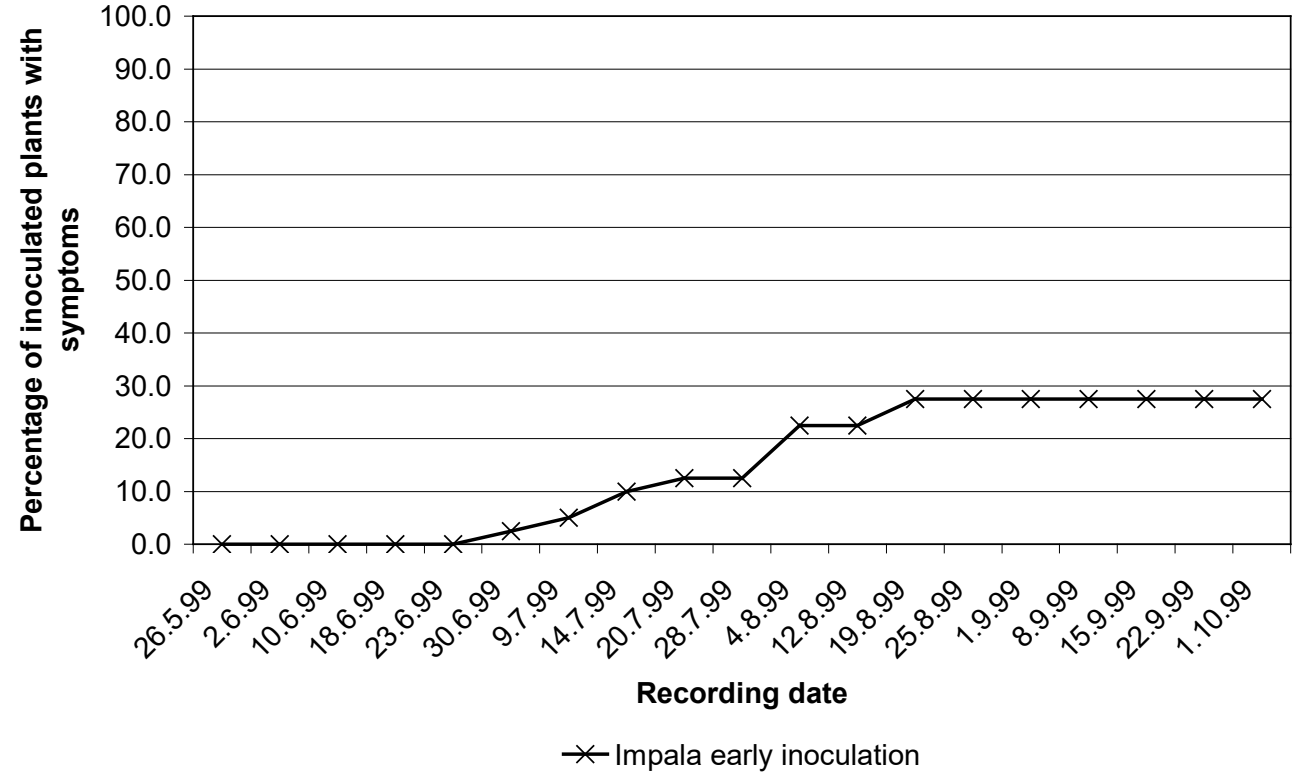


Figure 11. Percentage of plants inoculated with a combination of BWYV and CaMV showing symptoms

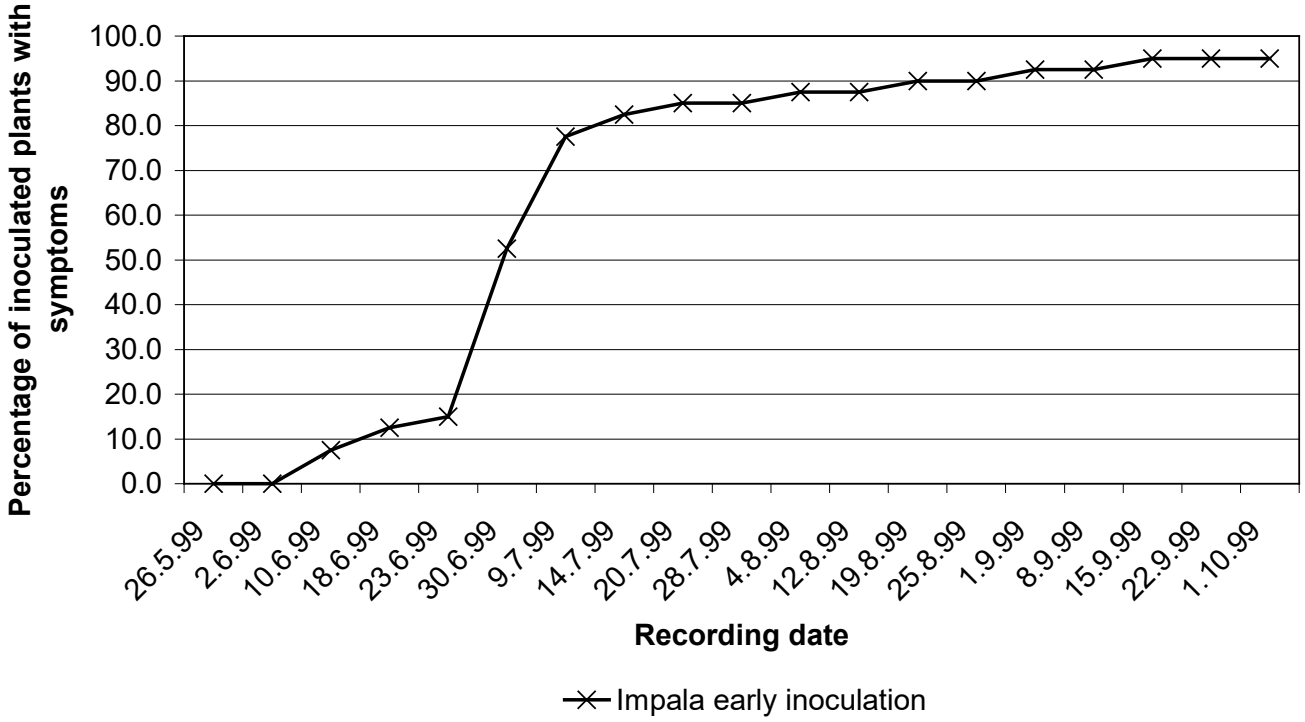
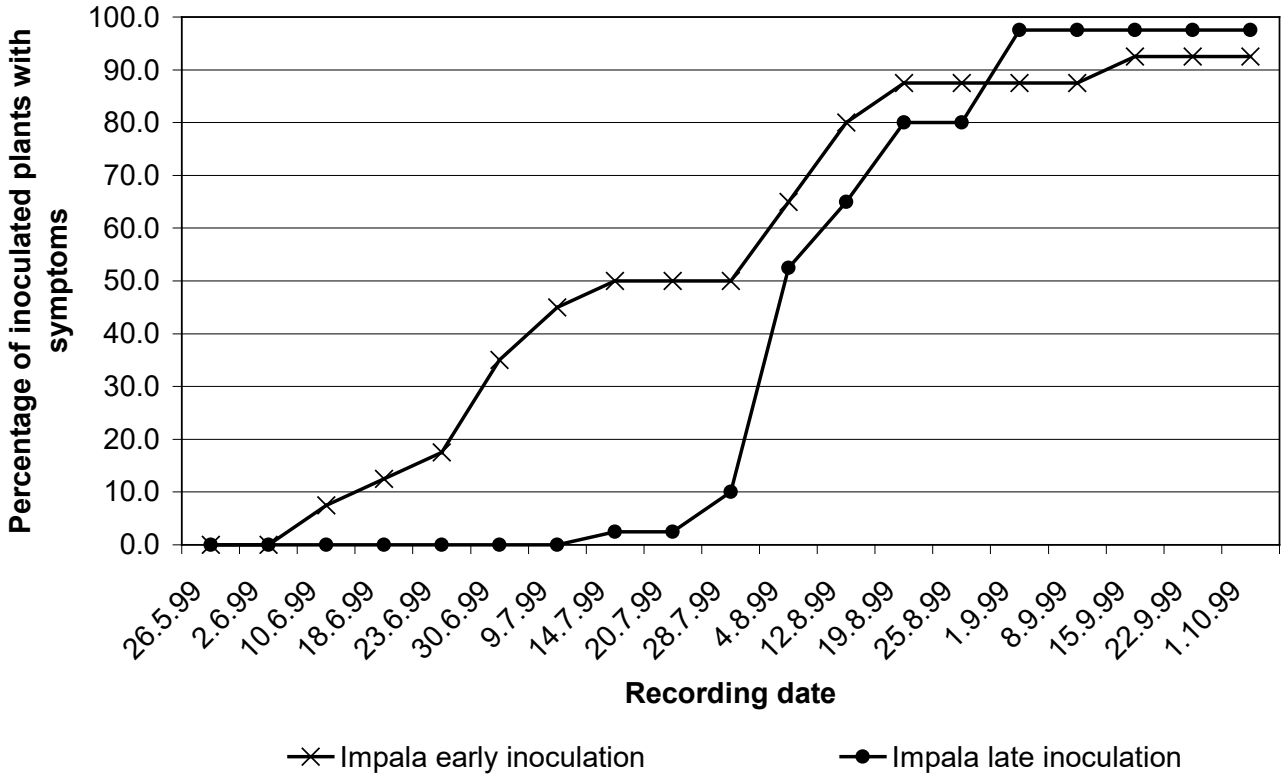


Figure 12. Percentage of plants inoculated with the combination of TuMV and CaMV showing symptoms



Concentrations of virus detected in plants prior to harvest

Statistical analysis of the ELISA data for BWYV in plants tested three weeks prior to harvest is shown in Table 4. Plants from all 3 virus treatments showed significantly higher mean ELISA values than uninoculated plants (no virus detected). The levels of BWYV detected in the BWYV + CaMV treatment was significantly greater than in other treatments, indicating that CaMV exacerbated BWYV infection. The level of BWYV detected in the BWYV + TuMV treatment was not significantly different from the levels detected with BWYV alone.

Table 4. Analysis of BWYV ELISA data at pre-harvest testing
Absorbance values at 405nm (A_{405}) transformed for statistical analysis using the formula:
$\log_e (A_{405} + 0.065)$

Treatment	Mean Transformed ELISA Values (A_{405})
BWYV	-1.149 **
BWYV + TuMV	-0.962 ***
BWYV + CaMV	-0.180 ***
Uninoculated	-2.901

** different from uninoculated treatment at 0.01 level of significance
*** different from uninoculated treatment at 0.001 level of significance

Statistical analysis of the ELISA data from plants inoculated with TuMV, sampled three weeks prior to harvest is shown in Table 5. The analysis showed a significantly higher ELISA value in the TuMV treatment compared to the uninoculated control (no virus detected), in the early-inoculated plants. In the late inoculated plants there were significantly greater levels of TuMV in the TuMV and in the TuMV + CaMV treatments than in the uninoculated controls (no virus detected). There were no significant differences between the levels of TuMV detected in the early and late inoculated plants. There was no BWYV + TuMV late inoculation treatment.

Table 5. Analysis of TuMV ELISA data at pre-harvest testing

Absorbance values at 405nm (A_{405}) transformed for statistical analysis using the formula:
$\log_e (A_{405} + 0.065)$

	Mean Transformed ELISA Values (A_{405})	
Treatment	**Early inoculation**	**Late inoculation**
TuMV + BWYV	-2.244	-
TuMV	-1.757 **	-1.968 *

TuMV + CaMV	-2.019	-1.456 ***
Uninoculated	-2.522	-2.593

* different from uninoculated treatment at 0.05 level of significance
** different from uninoculated treatment at 0.01 level of significance
*** different from uninoculated treatment at 0.001 level of significance

Statistical analysis of levels of CaMV prior to harvest was not possible due to the necessity of using inoculation to a susceptible host as a detection method. Incidence of the CaMV in early and late inoculated plants detected by this method (Table 6), showed the highest incidence in the CaMV + BWYV and the CaMV + TuMV treatments (virus detected in 57% of plants). The incidence data from the late inoculated plants, however showed no difference between the CaMV and CaMV + TuMV treatments. CaMV was detected in all plants of both treatments in the late inoculation. No infection by CaMV was detected in any uninoculated control plants. There was no BWYV + CaMV late inoculation treatment.

Table 6. Detection of CaMV in pre-harvest plants by inoculation to mustard (cv. Tendergreen) and confirmed by ELISA

Treatment	Early Inoculation			Late inoculation		
	Number tested	CaMV detected in	% Detection	Number tested	CaMV detected in	% Detection
CaMV + BWYV	40	23	57%	-	-	-
CaMV	40	18	45%	40	40	100%
CaMV + TuMV	40	23	57%	40	40	100%
Uninoculated	80	0	0%	40	0	0%

Head weight data

Statistical analysis of the head weight data recorded at harvest (Table 7) showed a significant reduction in mean head weight in virus-inoculated plants compared to uninoculated controls and a significant effect of inoculation time. Examination of the data for the early inoculation showed that CaMV infection, either alone or in combination with either BWYV or TuMV caused significant yield losses. BWYV alone also caused a significant reduction in yield. TuMV either alone or in combination with BWYV did not have a significant effect on head weight. In the late inoculations, both CaMV and CaMV + TuMV inoculated plants showed significant reductions in yield, whilst plants inoculated with TuMV alone did not. Furthermore, the yield losses recorded for the CaMV and CaMV + TuMV treatments were significantly greater for plants inoculated later compared to those inoculated pre-transplanting. This indicated that CaMV was the most important of the viruses in terms of yield loss and that potential losses may be greater if the infection occurs late in the growing season. BWYV could not be included in the late inoculations.

Table 7. Analysis of head weight data at harvest

REML analysis	Wald statistic	d.f.	Level at 5% Significance	Significance
Treatment	101.7	6	12.59	***
Inoculation time	16.8	1	3.84	***
Treatment. time	20.0	3	7.82	***

Treatment	Early Inoculation Mean Head Weight (kg)	Late Inoculation Mean Head Weight (kg)
BWYV	2.907 *	-
BWYV + CaMV	2.177 ***	-
BWYV + TuMV	3.229	-
CaMV	2.430 ***	0.867 ***
TuMV	3.354	3.582
TuMV + CaMV	2.852 *	1.379 ***
Uninoculated	3.702	3.677

* different from uninoculated treatment at 0.05 level of significance
*** different from uninoculated treatment at 0.001 level of significance

Internal symptoms

The incidence of internal cigar burn and tip-burn symptoms (Table 8) showed an association between the presence of cigar burn symptoms and TuMV inoculation. Furthermore, this association appeared to be stronger, both in the late inoculated plants and in heads that had been stored for longer. The lack of symptoms recorded in the 7 months assessment of TuMV late inoculated heads may be a consequence of the relatively small number of heads examined and the lower susceptibility of cultivar Impala to TuMV, relative to cultivar Polinius, noted in the previous year's experiment. The relatively low number of heads assessed was due to some heads being discarded at harvest as too damaged to store.

The incidence of tip-burn symptoms appeared to be associated predominantly with BWYV inoculation. CaMV may also be involved, but to a much lesser extent. There were more BWYV associated tip-burn symptoms after longer storage. The incidence of CaMV associated tip-burn symptoms did not appear to vary greatly with storage time. The difference in the numbers of plants assessed was due to heads being discarded at harvest as too severely damaged to store.

Table 8. Incidence of internal necrotic symptoms after storage

Incidence after 4 months in store

	Early Inoculation			Late Inoculation		
Treatment	**Number of Plants**	**Number with cigar burn**	**Number with tip-burn**	**Number of Plants**	**Number with cigar burn**	**Number with tip-burn**
BWYV	11	0 (0%)	1 (9%)	-	-	-
BWYV + CaMV	17	3 (18%)	10 (59%)	-	-	-
BWYV + TuMV	19	1 (5%)	1 (5%)	-	-	-
CaMV	13	0 (0%)	3 (23%)	7	0 (0%)	2 (29%)
CaMV + TuMV	14	1 (7%)	1 (7%)	3	11 (85%)	2 (15%)
TuMV	13	3 (23%)	0 (0%)	22	10 (45%)	0 (0%)
Uninoculated	38	0 (0%)	1 (3%)	17	0 (0%)	0 (0%)

Incidence after 7 months in store

	Early Inoculation			Late Inoculation		
Treatment	**Number of Plants**	**Number with cigar burn**	**Number with tip-burn**	**Number of Plants**	**Number with cigar burn**	**Number with tip-burn**
BWYV	19	0 (0%)	14 (74%)	-	-	-
BWYV + CaMV	17	0 (0%)	16 (94%)	-	-	-
BWYV + TuMV	20	4 (20%)	11 (55%)	-	-	-
CaMV	13	0 (0%)	2 (15%)	4	0 (0%)	1 (25%)
CaMV + TuMV	19	2 (11%)	3 (16%)	6	5 (83%)	0 (0%)
TuMV	16	4 (25%)	0 (0%)	12	0 (0%)	0 (0%)
Uninoculated	37	0 (0%)	1 (3%)	20	0 (0%)	0 (0%)

Statistical analyses of the mean symptom score data, (reflects both incidence and severity) of the internal disorders are presented in Tables 9 and 10. The negative value for the mean cigar burn score in the late inoculated CaMV heads assessed after 7 months in store (Table 8) is due to the statistical estimation of the value of missing data points in order to enable a statistical analysis to be conducted. This same feature of the analysis also accounts for the value of 0.002 for the late inoculated TuMV heads assessed after 7 months in store, where the incidence data (Table 7) showed no cigar burn detected.

The cigar burn data (Table 9) showed no significant effect of treatment in the early inoculated plants assessed after 4 months in store. However, early-inoculated heads assessed after 7 months in store, showed a significant level of cigar burn in heads from plants inoculated with TuMV alone. In heads from late inoculated plants assessed after 4 months of storage, significant levels of cigar burn were detected in both the TuMV and CaMV + TuMV treatments. In late inoculated heads assessed after 7 months in store, again significant levels of cigar burn were detected in the CaMV + TuMV treatment, however, no symptoms were recorded in heads inoculated with TuMV alone.

The data showed significant increases in cigar burn for late inoculated CaMV + TuMV treatments (after both 4 and 7 months in store) and for the late inoculated TuMV treatment (after 4 months in store) compared with the early inoculation of this treatment. Although CaMV did not produce significant levels of cigar burn when inoculated alone, significantly greater levels of cigar burn were seen in CaMV + TuMV late inoculated heads assessed after 4 and 7 months in store compared to those in heads from plants inoculated with TuMV alone. This indicated a significant exacerbation of TuMV-induced cigar burn symptoms by CaMV. As this effect was not seen in the early inoculated heads assessed after 7 months in store there was an indication of an interaction between treatment and infection time.

Table 9. **Mean internal cigar burn symptom scores in stored heads**

	4 Months in store		**7 Months in store**	
Treatment	**Early Inoculated**	**Late Inoculated**	**Early Inoculated**	**Late Inoculated**
BWYV	0.000	-	0.005	-
BWYV + CaMV	0.116	-	0.005	-
BWYV + TuMV	0.125	-	0.155	-
CaMV	0.003	0.000	0.002	-0.055
CaMV + TuMV	0.165	1.512 ***	0.153	0.833 *
TuMV	0.175	0.490 ***	0.980 ***	0.002
Uninoculated	0.000	0.000	0.005	0.005

* different from uninoculated treatment at 0.05 level of significance
*** different from uninoculated treatment at 0.001 level of significance

The tip-burn data (Table 10) showed a significantly higher level of tip-burn in the BWYV + CaMV treatment in heads assessed after 4 months in store and in all of the treatments involving BWYV in heads assessed after 7 months in store relative to the uninoculated heads. There was no significant tip-burn seen in treatments that did not involve BWYV from either early or late inoculated plants at either assessment.

The tip-burn data showed an exacerbation effect involving CaMV similar to that seen with cigar burn. Significantly greater levels of tip-burn were seen in heads from plants inoculated with BWYV + CaMV than in heads from plants inoculated with BWYV alone at both assessments. There is currently no information available on the effect of BWYV infection timing on symptom severity. The tip-burn data also indicated the possibility of a second interaction, between BWYV and TuMV. BWYV + TuMV infected heads assessed after 7 months in store had significantly less tip-burn than the heads from plants inoculated with BWYV alone. This indicated that TuMV infection might ameliorate the effect of BWYV-induced tip-burn.

Table 10. Mean internal tip-burn symptom scores in stored heads

Tip-Burn	**4 Months in store**		**7 Months in store**	
Treatment	**Early Inoculated**	**Late Inoculated**	**Early Inoculated**	**Late Inoculated**
BWYV	0.044	-	0.937 ***	-
BWYV + CaMV	1.102 ***	-	1.412 ***	-
BWYV + TuMV	0.027	-	0.481 **	-
CaMV	0.291	0.150	0.142	0.085
CaMV + TuMV	0.044	0.221	0.148	0.000
TuMV	0.002	0.000	0.057	0.086
Uninoculated	0.023	0.002	0.018	0.057

** different from uninoculated treatment at 0.01 level of significance
*** different from uninoculated treatment at 0.001 level of significance

Predictive tests

Three weeks prior to harvest, all plants were tested for the presence of the viruses with which they were inoculated. Uninoculated control plants were tested for all three viruses. BWYV and TuMV inoculated plants were tested by ELISA and CaMV inoculated plants were tested by inoculation to susceptible indicator plants followed by ELISA testing of the indicator plants. The results (Table 11) showed that TuMV was detected in approximately 61% of the TuMV inoculated plants that developed cigar burn and that BWYV was detected in approximately 86% of the BWYV inoculated plants that developed tip-burn. This demonstrated effective and rapid (3 days) pre-harvest tests for BWYV and TuMV infection.

Table 11. Virus detection prior to harvest in heads which later developed internal necroses.

Cigar burn symptoms

Treatment	Number of plants with cigar burn	Number with BWYV	Number with CaMV	Number with TuMV
BWYV	0	0	nt	nt
BWYV + CaMV	3	3 (100%)	0 (0%)	nt
BWYV + TuMV	5	4 (80%)	nt	3 (60%)
CaMV	0	nt	0	nt
CaMV + TuMV	19	nt	4 (21%)	11 (57%)
TuMV	17	nt	nt	11 (65%)
Uninoculated	0	0 (0%)	0 (0%)	0 (0%)

Tip-burn symptoms

Treatment	Number of plants with tip-burn	Number with BWYV	Number with CaMV	Number with TuMV
BWYV	15	15 (100%)	nt	nt
BWYV + CaMV	30	30 (100%)	20 (67%)	nt
BWYV + TuMV	12	7 (58%)	nt	2 (17%)
CaMV	8	nt	1 (13%)	nt
CaMV + TuMV	6	nt	0 (0%)	2 (33%)
TuMV	0	nt	nt	0
Uninoculated	2	0 (0%)	0 (0%)	0 (0%)

nt - not tested for a particular virus

Post storage virus detection and recovery

The samples taken from the heads at assessment showed no cross contamination of the viruses from different treatments. BWYV was detected by ELISA only, TuMV was detected by ELISA and by inoculation (samples with symptoms) to susceptible indicator plants followed by ELISA of indicator plants. CaMV was detected by inoculation to susceptible indicator plants followed by ELISA of indicator plants. No virus was detected in samples from uninoculated plants, including samples from the two heads with tip-burn. The data (Table 12) showed detection of BWYV in 86% of BWYV inoculated heads, detection of CaMV in 67% of CaMV inoculated heads and detection of TuMV in 23% of TuMV inoculated heads. Detection of virus in samples from heads that had developed symptoms was greater than the overall levels of detection. BWYV was detected in 100% of symptomatic heads tested, CaMV in 79% of symptomatic heads and TuMV in 57% of heads which had symptoms. The

appropriate viruses were therefore detected in heads where symptoms had been induced by virus inoculation.

Table 12. Detection of virus from heads after storage

Virus Inoculated	Number of heads tested	Number of heads with symptoms	Number of heads in which virus was detected (Number of these heads which had symptoms)
BWYV	108	58	93 (58)
CaMV	124	59	83 (47)
TuMV	159	54	37 (31)
Uninoculated	112	2	0 (0)

OBJECTIVE 3
PHYSIOLOGICAL/VIROLOGICAL STUDIES

Material & Methods

Hydroponic experiments in glasshouse
Details of the design and layout of the hydroponic system were provided in the annual project report for 1999 and will not be repeated here. In brief, the system comprises 8 benches with 16 plant stations on each bench. The containers are supplied with nutrient solution from 4 separate tanks. These containers are located randomly on the benches with respect to their tank connection. Nutrient treatments can be allocated to different tanks in different experiments, but the randomisation is fixed. Treatments such as variety can be randomised anew for each experiment.

Two experiments were started during the last reporting year.

Experiment, June to December 1999
Previous experience had shown that better plants are obtained if seeds are germinated directly in the hydroponic system so that the seedlings experience a nutrient solution environment from an early stage rather than the shock of movement from a substrate-based system. The potential for introducing contamination into the hydroponic system is also reduced.
The main hydroponic system was in use until mid May from a previous experiment. In addition to the usual extensive cleaning and decontamination procedures, we undertook major replacement of the “drainage” pipework, which returns nutrient to the tanks, with larger bore pipe to minimise blockages. In order to prevent further delay of the next experiment a hydroponic “nursery was constructed to start the seedlings. The nursery was also used to permit quarantined infection of some plants with virus (see later).

Seeds of Dutch White cabbage (*Brassica oleraceae* L.), cv. Impala were sown on sterilized moist germination paper in clear plastic boxes on 18 June 1999. They were transferred to the nursery tanks on 24 June, when the nursery system was ready. The nutrient solution was a quarter concentration of the “full nutrient” regime specified in Table 1 of the project report for 1998 (year 2).
On 12 July some of the plants were moved to similar nursery tanks in a different glasshouse compartment to carry out infection with beet western yellows virus (BWYV) using aphids to inoculate the plants. These plants were sprayed with “Dovetail” to kill the aphids on 19 July. An equivalent spray was also applied to the uninoculated plants to ensure uniformity of treatment and all plants were treated with “Dovetail” for a second time on 2 August. All plants were transferred from the nursery system to the main hydroponic system on 20 July and infection of plants with cauliflower mosaic virus (CaMV) or turnip mosaic virus (TMV) was done on 21 July by mechanical abrasion.

Nutrients were replaced on the basis of weekly analyses of the solutions (Figure 13). Replenishment was initially to a quarter of full strength. From 13 August, a target of half-strength was used and from 15 October concentrations were raised using full strength as the target.

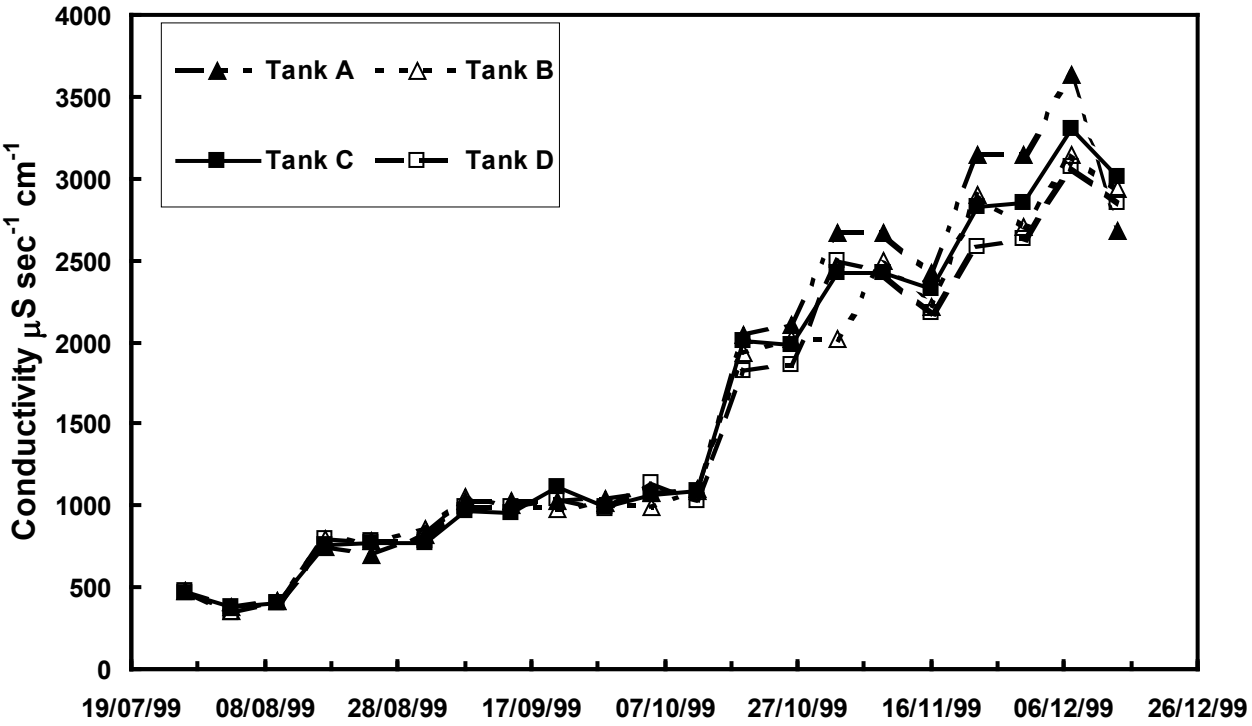


Figure 13. Conductivity of nutrient solution. Hydroponic experiment 1999

Calcium replacement ceased for two of the supply tanks from 12 October, thus imposing calcium stress on half of the plants (Figure 14). This was maintained until the 14 and 15 December when heads were harvested and stored at 3°C. On 28 and 29 February 2000, the heads were weighed, examined for incidence of internal necrosis and samples removed for calcium and virus analysis. From 7 October to the end of the experiment, daylight was supplemented with

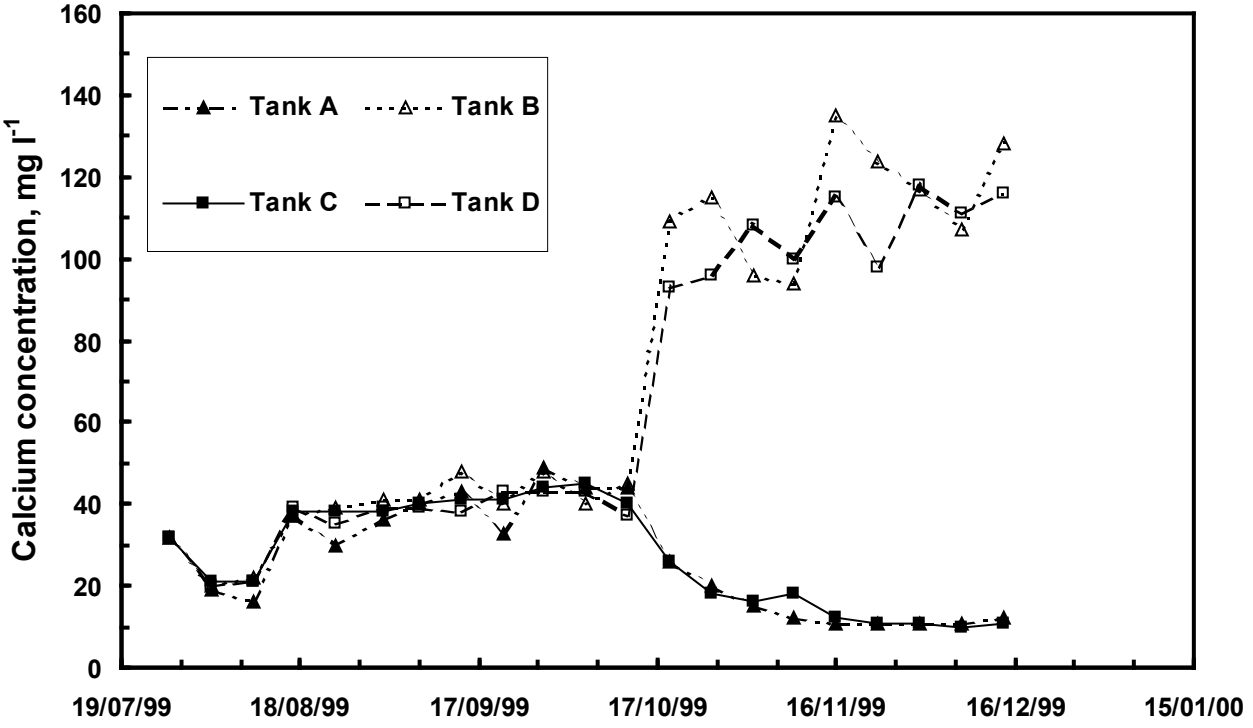


Figure 14 Calcium concentration of nutrient solution. Hydroponic experiment 1999

12 Wm^{-2} of irradiance from 16 high-pressure sodium lamps to maintain growth. Reciclean, a proprietary agent for disinfecting irrigation systems was added to the nutrient solution at a concentration of 25ppm to inhibit root infection with *Pythium*. It is used as a mixture of formic acid and hydrogen peroxide, which forms the active ingredient, performic acid. During July and August, additions were made at approximately weekly intervals. This increased to four days per week from September to the end of the experiment.

On 8 November 1999 plants were sampled for virus detection prior to final harvest. Samples of leaf material were also taken from plants inoculated with CaMV and from uninoculated plants for investigation of PCR based detection for CaMV on 2 December 1999. Heads were harvested and weighed on 14 and 15 December 1999 and placed in cold storage at HRI-Wellesbourne.

For PCR based detection of CaMV from cabbage plants prior to harvest, DNA was extracted from leaf samples according to the method of Covey et al. (Covey S.N., Noad R.J. Al-Kaff N.S. and Turner D.S. "Caulimovirus Isolation and DNA extraction" in Methods in Molecular Biology (1998) Vol 81: Plant Virology Protocols: from virus isolation to transgenic resistance pp 53-60). The DNA was amplified using 3 sets of primer pairs, which amplified fragments from the coat protein gene, the RNA polymerase gene and the 19s region. Amplification products were electophoresed on a 1% agarose gel and visualised with ethidium bromide under ultraviolet light.

Heads were removed from storage on 28 February 2000 and assessed for internal symptoms using the scoring system given in Table 1). Samples were taken for detection of virus using ELISA for BWYV and TuMV and inoculation to susceptible indicator plants followed by ELISA of the indicator plants for CaMV. Samples of calcium analysis were also taken from all heads.

Experiment, March to September 2000
In this experiment, plants were infected only with BWYV and then subjected to calcium withdrawal during head development. This was similar to the 1999 experiment, but used only one virus treatment. This permitted greater replication and thus more confidence in interpreting the results.
The hydroponic system and all removable components were thoroughly disinfected twice with Reciclean at 100ppm between experiments. All surfaces in the compartment were sprayed with another disinfectant, Jet 5 (active ingredient, peroxyacetic acid).
Seeds of cv Impala were sown directly into the nutrient containers on 24 March 2000 (3 seeds per container). On 10 and 11 of April the germinated seedlings were thinned to one per container.
Infection of plants with BWYV was done on 26 April using aphid vectors to introduce the virus. To avoid inadvertent inoculation of control plants by the aphids, these plants were removed from the main compartment to a system based on the “nursery” tanks in a different compartment. It had been hoped to avoid this by enclosing the aphids in clip cages. However, this proved impractical, so isolation of the different treatments became necessary. On 4 May, plants in both compartments were treated with “Dovetail” and the uninoculated plants returned to the main system on 5 May.

Reciclean was added to the system once only, on 24 May. After its addition the plants showed a dramatic wilting response, from which they recovered within 24 hours. Although this response had been observed in the previous experiment it had been less dramatic and the plants had eventually not reacted to additions of Reciclean. However, we became aware that Filex (active ingredient, propamocarb hydrochloride), a fungicide that is effective against *Pythium*, had been used in some nutrient-based systems. Our own tests with young cabbage plants suggested that there was no adverse effect of this product when added at 2.5ml per 10 litres to a re-circulating hydroponic system. The presence of *Pythium* spores was not detected in samples taken from the system on 13 June and 14 July. However a badly wilted plant was observed on 6 July and presumed to be affected by *Pythium*. Filex was added to all tanks on 6 and 27 July. External symptoms were recorded weekly.

Results & Discussion

Hydroponic experiment 1999
The purpose of this experiment was to examine whether symptoms of internal necrosis in cabbage heads are affected by the interaction of infection with beet western yellows virus (BWYV) cauliflower mosaic virus (CaMV) or turnip mosaic virus (TMV) and the withdrawal calcium from the nutrient medium.

Table 13. Mean head fresh weights (g) at harvest for plants from 1999 hydroponic experiment.

Treatment	Control	Calcium withdrawn
Uninoculated	1017	1012
BWYV	1000	668
TuMV	1072	829
CaMV	1004	982

This experiment provided 115 heads (from a maximum possible of 128) with an average fresh weight of 0.95kg at harvest. Heads subjected to calcium withdrawal had a smaller mean fresh weight than those not treated in this way (Table 13). This was almost entirely due to interaction with the virus treatments. Mean head fresh weight for all virus treatments was smaller than for uninoculated plants, with BWYV having the greatest effect.

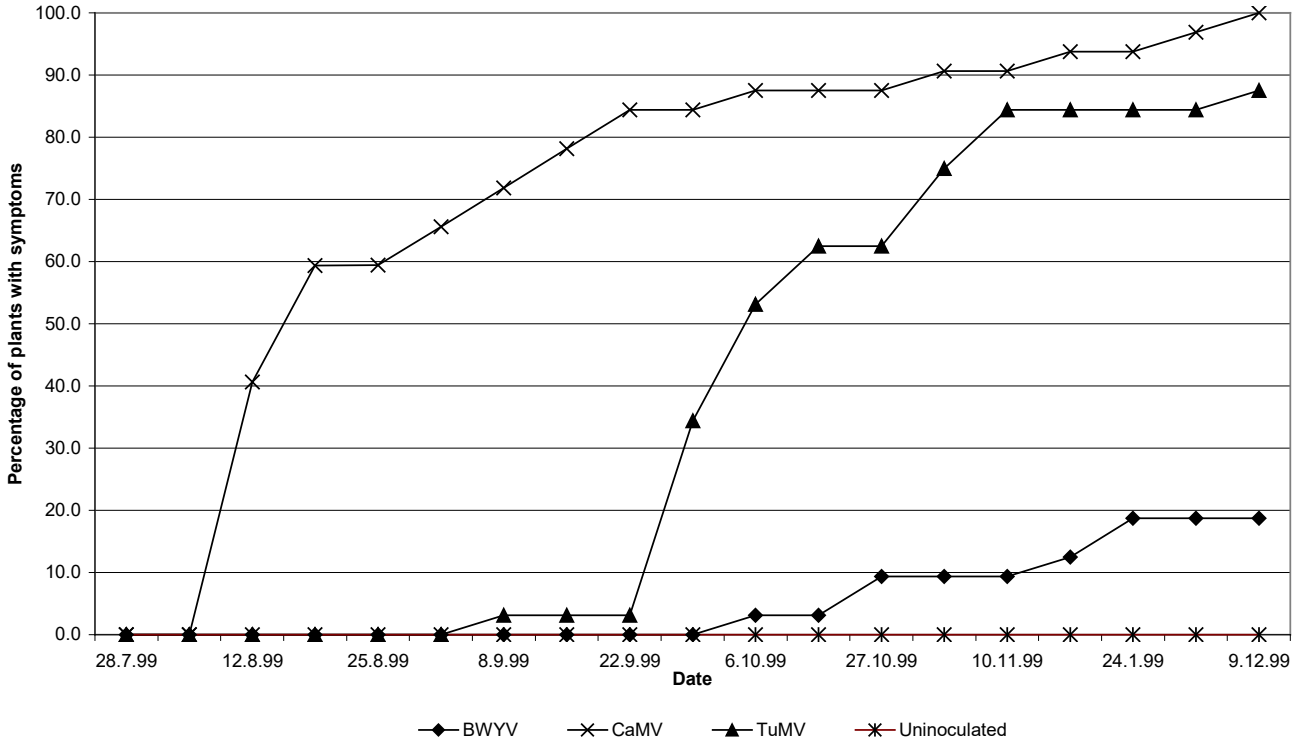
External virus symptoms

The data (Figure 15) showed a similar pattern to that observed in the Tygan house experiment (objectives 1 and 5). External symptoms in CaMV inoculated plants, characterised by vein clearing, progressing to chlorosis of outer leaves appeared 3 weeks after inoculation and increased rapidly to approximately 85% incidence of inoculated plants, the incidence then increased steadily during the experiment, reaching 100% by harvest. External necrotic spotting on TuMV inoculated plants also followed a pattern similar to that seen in the Tygan experiment. The symptoms first appeared 7 weeks after inoculation but did not seem to increase for 2 weeks. The symptoms then showed a rapid increase in

incidence reaching a maximum incidence of approximately 87% by harvest, 16 weeks after inoculation.

BWYV inoculated plants showed more external symptoms in the hydroponic experiment than was the case in the Tygan experiment. This may have been a consequence of the hydroponic environment. The symptoms appeared 12 weeks after BWYV inoculation. The incidence of symptoms increased steadily over the next 8 weeks to reach a maximum level of just less than 20%. As in the case of external symptoms in the Tygan experiment, the symptoms were of the chlorotic type; similar to those observed on the CaMV inoculated plants rather than necrotic.

Figure 15. Progression of external symptoms in the hydroponic experiment



Internal symptoms

Heads were assessed after 3 months in store. The incidence of symptoms is shown in Table 14. Plants inoculated with BWYV developed tip-burn but not cigar burn symptoms and plants inoculated with TuMV developed cigar burn but not tip-burn symptoms. Plants inoculated with CaMV developed both tip-burn and cigar burn. This is in contrast to the Tygan experiment, where CaMV alone produced no cigar burn and very little tip-burn and may be a consequence of the artificial growth conditions imposed using the hydroponic system. The level of incidence of tip-burn induced by CaMV inoculation was lower than that induced by BWYV in both the high and low calcium regimes. In contrast, the incidence

of CaMV induced cigar burn was the same as for TuMV in the high calcium regime and greater than for TuMV in the low calcium regime.

Heads from the BWYV inoculated plants showed no difference in incidence of tip-burn between calcium regimes. TuMV inoculation produced fewer heads with cigar burn under the low calcium regime than the high calcium regime. There was no effect of calcium regime on incidence of CaMV-induced cigar burn, and a slight reduction in incidence of CaMV-induced tip-burn in the low calcium regime.

Table 14. Incidence of internal necrotic symptoms in hydroponically grown plants after storage

	Incidence after 3 months in store					
	High calcium			Low calcium		
Treatment	Number of Plants	Number with cigar burn	Number with tip-burn	Number of Plants	Number with cigar burn	Number with tip-burn
BWYV	16	0 (0%)	5 (31%)	16	0 (0%)	5 (31%)
CaMV	16	4 (25%)	3 (19%)	16	4 (25%)	2 (12%)
TuMV	16	4 (25%)	0 (0%)	16	2 (12%)	0 (0%)
Uninoculated	16	0 (0%)	0 (0%)	16	0 (0%)	0 (0%)

The mean symptom scores (Table 15) combine both incidence and severity of symptom. A statistical analysis of the data indicated that BWYV inoculation induced a significant level of tip-burn in both high and low calcium regimes, relative to the uninoculated controls. The levels of tip-burn recorded with CaMV inoculation were not statistically significant. CaMV inoculation caused a significant level of cigar burn under the high calcium regime, relative to the uninoculated controls but not under the low calcium regime. This indicated a

Table 15. Mean symptom scores for cigar burn and tip-burn from hydroponically grown plants after 3 months in store

	Cigar burn		Tip-burn	
Treatment	High calcium	Low calcium	High calcium	Low calcium
BWYV	0.000	0.000	0.438 *	0.469 *
CaMV	0.344 *	0.125	0.188	0.125
TuMV	0.313	0.188	0.000	0.000
Uninoculated	0.000	0.000	0.000	0.000

* different from uninoculated treatment at 0.05 level of significance

calcium / virus interaction involved with cigar burn. TuMV did not produce a significant level of cigar burn relative to the uninoculated controls (l.s.d. 0.318), despite the same incidence of symptoms under the high calcium regime as produced by CaMV interaction. This may have been due the short period in store, since evidence from the Tygan experiment (objectives 1 and 5) indicated that the severity of TuMV induced cigar burn increased with storage time.

Predictive tests

Plants were tested for the presence of the inoculated viruses 5 weeks prior to harvest. ELISA tests were performed for BWYV and TuMV. PCR tests and inoculation to susceptible indicator plants, followed by ELISA of the indicator plants were performed for CaMV. The results (Table 16) showed that the methods employed gave good levels of detection prior to harvest in plants that developed symptoms in storage. Detection levels for CaMV were higher in heads that developed tip-burn than in heads that developed cigar burn.

Table 16. Pre-harvest virus detection in heads which developed internal necroses during storage.

Cigar burn symptoms

Treatment	Number of plants with cigar burn	BWYV detected in	CaMV detected in	TuMV detected in
BWYV	0	0	0	0
CaMV	8	nt	6 (75%)	nt
TuMV	6	nt	nt	5 (83%)
Uninoculated	0	0	0	0

Tip-burn symptoms

Treatment	Number of plants with tip-burn	BWYV detected in	CaMV detected in	TuMV detected in
BWYV	10	8 (80%)	nt	nt
CaMV	5	nt	5 (100%)	nt
TuMV	0	0	0	0
Uninoculated	0	0	0	0

Virus detection after storage

Samples from all plants after storage were tested for the viruses with which they were inoculated. Uninoculated control plants were tested for all three viruses. BWYV was detected by ELISA alone, TuMV was detected by ELISA and by inoculation (samples with symptoms) to susceptible indicator plants followed by ELISA of indicator plants. CaMV was detected by inoculation to susceptible

indicator plants followed by ELISA of indicator plants. The data (Table 17) showed approximately 30-50% detection from inoculated heads after storage for all viruses. Virus detection in heads that had developed symptoms was higher than the overall detection level (approximately 50-75%). The appropriate viruses were therefore detectable in heads in which symptoms had been induced by virus inoculation.

Table 17. Detection of virus from heads after storage

Virus Inoculated	Number of heads tested	Number of heads with symptoms	Number of heads in which virus was detected (Number of these heads that had symptoms)
BWYV	26	10	12 (4)
CaMV	30	13	10 (4)
TuMV	30	6	17 (6)
Uninoculated	30	0	0

Hydroponic experiment 2000

This experiment was set up to confirm the findings of the previous hydroponic experiment and other experiments, that there seemed to be an association between the presence of BWYV and tipburn type symptoms in cabbage heads. This experiment is still ongoing at the time of writing.

Growth and health of the plants in this experiment has been exceptionally good. A large proportion (71%) of the plants developed external symptoms of calcium deficiency (marginal necrosis and “drawstring” (Figure 16) on younger leaves from early July well before calcium withdrawal was begun. Many of the plants had developed large heads by late July. On 10 July average head diameter was just over 9 cm with many individuals of up to 15 cm.

Figure 16. Illustration of “drawstring” and marginal necrosis on plants from hydroponics experiment in 2000

Subsequent expansion has resulted in split heads on approximately 25% of the plants.

The plants were provided with the usual nutrient solution regime, beginning at quarter strength for seedlings and gradually increasing to full strength as the plants grew (as illustrated in Figure 13 for the 1999 experiment). Calcium concentration (Figure 17) was maintained at full strength for those plants to be supplied continually with calcium and half strength for plants from which calcium was to be withheld. Calcium replacement was suspended for these plants after 18 July.

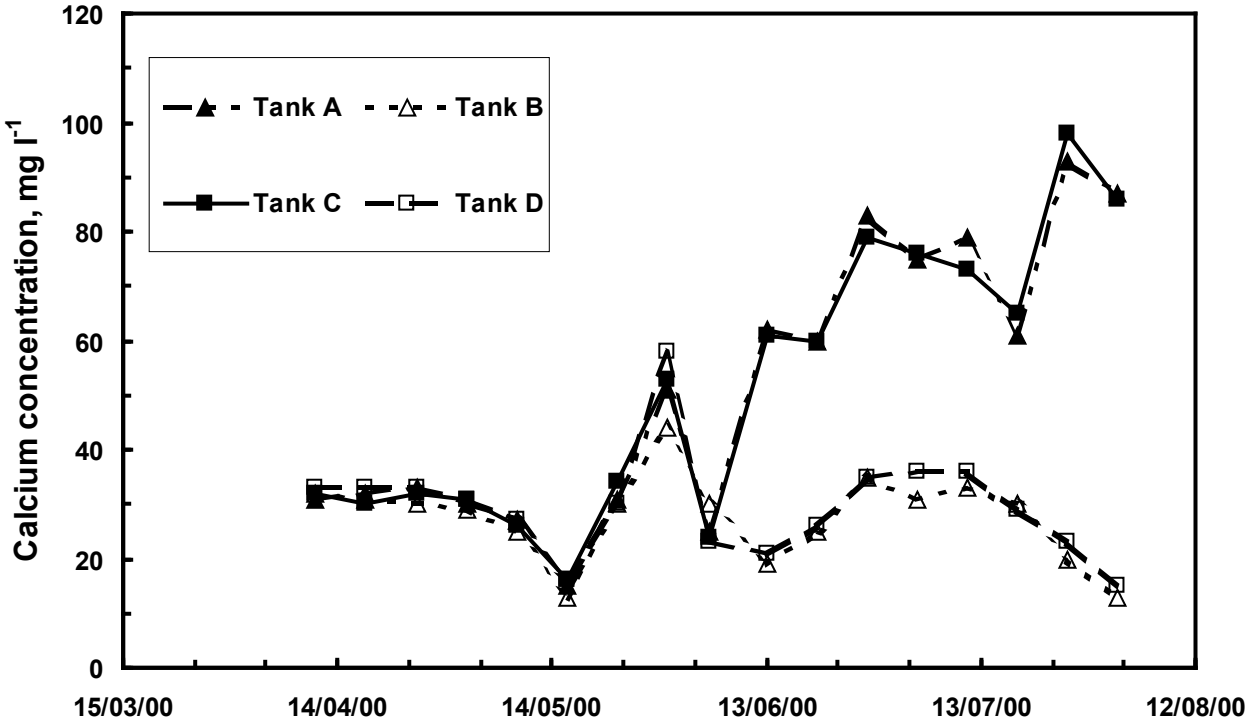


Figure 17. Calcium concentration of nutrient solution. Hydroponic experiment 2000

OBJECTIVE 4
AGRONOMIC STUDIES

Materials and Methods

A fan design (parallel row type) density experiment with 18 fans and 50 m x 80 m in size was established with two varieties, Albion a widely-grown Industry standard and Impala. These were planted at Kirton on 10/11 May 1999. The crop was grown to Industry protocols provided by Univeg.

The crop was harvested on 6 to 11 October, head weights were recorded before plants were put into store at Univeg. Since the industry standard specifies a minimum head diameter (at widest point) (especially important for automatic coring) rather than a minimum weight a weight/diameter relationship (i.e. mean density measurement) was produced from samples from a single fan for each variety. This relationship was then imposed on the weight data from the other fans.

In order to gather information about 'processability' of the material, the processors in the Consortium were provided with 1 head from each of 2 varieties and each of 6 spacings (12 heads in all). Each processor received samples from a different replicate fan, thus blocking "processor variability" with variation between replicate fans. The heads sent to processors were removed from store at Univeg assessed at Kirton before being sent to processors. This enabled split heads (from the assessments) to be sent along with the whole heads for "processing" to allow the processors to make judgements about the size of core etc (important for those processors using automated coring).

The following records were made: diameter of head, head weight before processing, weight of useable material (after trimming and coring), whether or not shred length acceptable (by visual inspection of head).

The processors also provided information on leaf texture, shred thickness, whether head accepted or rejected for processing and the reasons.

Results

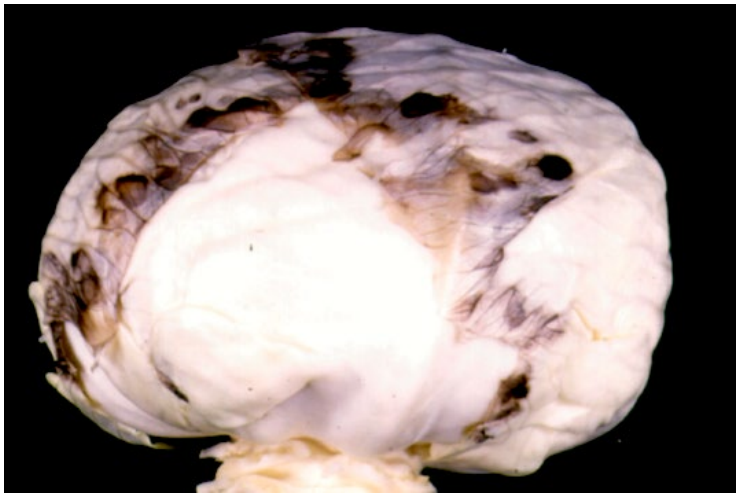
Head weight increased linearly with increasing space per plant from 2.5 kg at 50 cm x 50 cm to 5.5 kg at 75 cm x 75 cm spacing. The response was similar in both varieties. Albion had fewer internal defects than Impala. In Impala, Cigar burn increased with wider spacing and it was worse following storage. Samples are being analysed for Ca^{2+} concentration and data from the processors on 'processability' is being collated.

APPENDIX

Figure 18.

Cigar burn in cv. Impala inoculated with TuMV

Figure 19.



Tip burn in cv. Impala inoculated with BWYV + CaMV